GROWTH OF LITTER FUNGI IN A FOREST SOIL

T. F. HERING

Nature Conservancy, Merlewood Research Station, Grange-over-Sands, Lancs., England

Introduction

It is recognised that the isolation of a fungal species from a substrate in soil is not necessarily evidence for its participation in the process of breakdown; it may be present through contamination, or as a resting stage after the end of active growth. The present limited investigation was made to test the possibility of using a simple test to determine the stage of decomposition during which particular fungi are most active.

For the present work five species of fungi were studied. Their occurrence in oak leaves during the two years after leaf-fall suggested that their roles in breakdown differed considerably. They were tested against four distinct layers from a single forest soil. The measure used for assessing fungal growth was mycelial extension, since this attribute is simply measured and is advantageous in a soil habitat (GARRETT, 1956).

Site and Materials

The source of the L, F, H, and A layer materials used for the experiments was a wood of *Quercus petraea* in Grisedale, Lancs. (Nat. Grid Ref SD/335907) on a very shallow soil overlying slate. An understory is lacking and the ground flora consists almost solely of *Polytrichum formosum*. The soil is a brown forest soil of low base status, and a moder type of humus is developed (Table 1).

H layer and A layer material were sieved (1.5 cm mesh) to remove roots and the larger stones. All the materials were freshly collected, and kept at a natural moisture content for the duration of the experiments.

TABLE 1. Details of the experimental soil

Layer	Depth	Mean pH	Remarks
L	3—0 cm	4.3	Almost entirely oak leaves
F	0—2 cm	4.1	Dark brown, patchily distributed
H	2—5 cm	3.8	Blackish, with abundant roots
A	5—10+ cm	4.0	Brown, humus-stained above, very stony; lower boundary indistinct

The test fungi. The five test fungi were *Trichoderma viride* PERS. ex FR., *Mucor hiemalis* WEHMER, *M. ramannianus* MÖLLER, *Cladosporium herbarum* LINK ex FR. and *Polyscytalum fecundissimum* RIESS. The isolates were all obtained from leaf-litter at Grisedale, Lancs.

Preliminary observations on the test fungi. (a) Occurrence in leaf-litter. A series of isolations from fallen oak leaves, by agar plating of washed fragments, gave the following distributions:—
T. viride showed an increasing incidence on oak leaves during the two-year period.
M. hiemalis was first isolated six months after leaf-fall and persisted to the end of the second year.
M. ramannianus was not initially present, but appeared eight months after leaf-fall and increased slowly to the end of the second year.
C. herbarum was isolated in variable quantity throughout the two-year period.
P. fecundissimum was dominant on freshly-fallen oak leaves, and declined to nothing over a period of twenty months.

(b) Occurrence on living leaves. A list of about twenty species isolated from living oak leaves in the wood in the summer and autumn of 1960 included *C. herbarum*, but not *P. fecundissimum*.

(c) Colonisation in soil. In an attempt to assess the extent to which the test fungi colonised new material in soil, 4 mm discs of sterilised oak lamina were incubated on the soil materials (L layer omitted) for two weeks at a mean temperature of 11°C. The discs were then washed with mechanical shaking and plated on agar. The percentage occurrence of the test fungi on samples of fifty discs is recorded in Table 2.

TABLE 2. Percentage colonisation of 50 test discs by five test species of fungi

Species	Soil layers F	H	A
Trichoderma viride	68	88	10C
Mucor hiemalis	8	0	0
Mucor ramannianus	2	6	4
Cladosporium herbarum . . .	0	0	0
Polyscytalum fecundissimum .	0	0	0

Experimental Methods and Results

Extension was measured over the surface of oak-leaves from the litter layer, which were sterilised with propylene oxide (48 hr exposure; 96 hr de-gassing). They were inoculated with the test fungus by placing a 4 mm agar disc, cut from a plate culture on Czapek-Dox agar, on the underside near the apex. Oak leaves were chosen for the test as they provide a nutrient base as closely akin as possible to what is available in the soil. The test leaves were enclosed in sheets of cellophane (British Cellophane Company P.T. 300-thickness 20 μ; boiled before use), to shield them from colonisation after burial by organisms from the surrounding medium. KERR and FLENTJE (1957) have shown that cellophane of this thickness was readily permeable to substances affecting the growth of *Pellicularia filamentosa*, and DOBBS and HINSON (1953) showed that cellophane transmitted their "soil mycostatic factor".

The leaves in their cellophane covers were buried in the appropriate media (L, F, H, and A layer materials from the test site) in seedling boxes. L and F layer materials were compressed by a layer of sand 3 cm. deep, to ensure close contact between the cellophane and the surrounding medium. In a fifth, the control treatment, the surrounding medium was sterile acid-washed sand at 50% of its water-holding capacity (mean pH 5.7). For the two fastest-growing fungi, *T. viride* and *M. hiemalis* the incubation period was 4 days; for the remaining species 8 days.

To assess mycelial extension, a number of direct and indirect methods were tried. Direct examination of the leaves or cellophane proved tedious

and very unreliable; hyphae failed to adhere consistently to the cellophane, and were only observed with great difficulty against the leaf surface. Leaves were cut up and incubated in damp chambers, but some of the test fungi did not make visible growth. Finally an agar plate method was adopted, as follows: — using sterile 4 mm cork borers, a row of discs was cut out of each leaf, the centres of the discs being specified distances from the site of inoculation. For the two faster growing species, the interval chosen was 1 cm; for the remaining species 0.5 cm. The discs were transferred to agar plates, and the extent of spread was revealed by the appearance of characteristic colonies. In the presentation of results, extension is expressed in mm per day. The results in Fig. 1 are the means of 24 replicates.

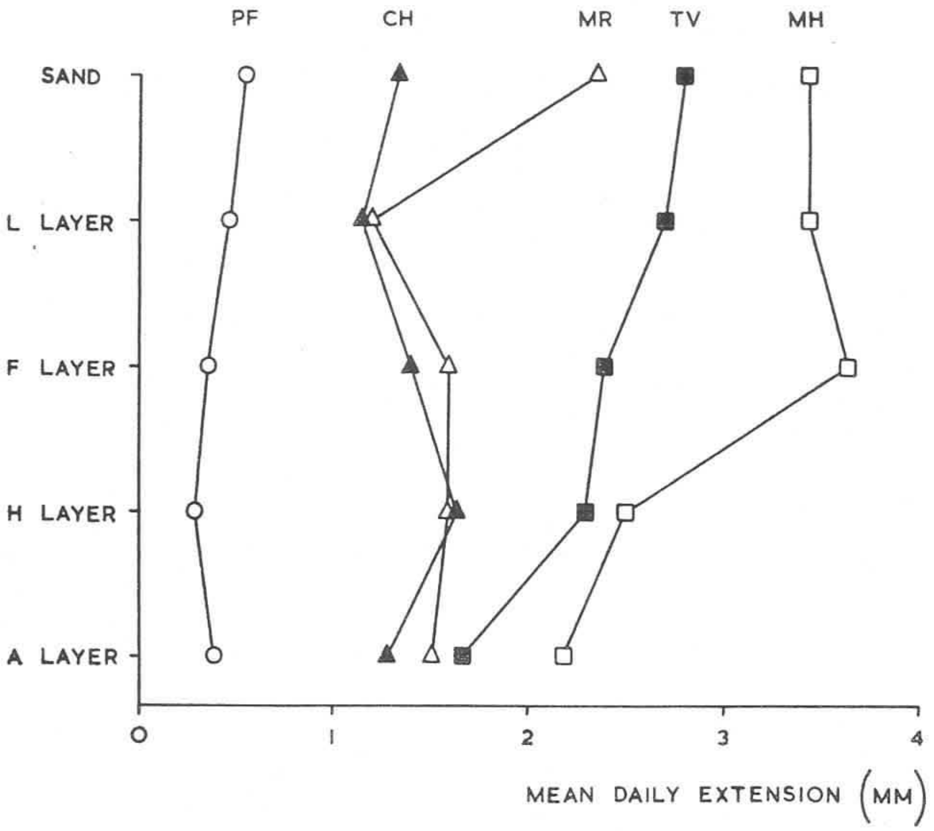

Fig. 1. Daily rates of extension of five fungi in four soil layers and a sand control (means of 24 replicates).

PF. *Polyscytalum fecundissimum* CH. *Cladosporium herbarum*
MR. *Mucor ramannianus* MH. *Mucor hiemalis* TV. *Trichoderma viride.*

Discussion

The aim of the above experiment was to find a simple method by which the effects of soil environment on a single fungus might be studied. It is not claimed that the test environments exactly match conditions in the soil itself, but they may be close enough for conclusions to be reached which apply to the soil.

The estimates of mycelial extension must be regarded as analogous to a mycelium spreading over a colonised substrate and through soil spaces. No very pronounced effects have been demonstrated; the greatest decrease in mean daily extension, compared with the control, was of the order of 50 %, and this did not prove statistically significant. It seems, therefore, that mycelial extension over a substrate is less profoundly affected by a soil environment than is spore germination, studied by DOBBS and HINSON (1953), who found that spores capable of germinating in pure water fail to do so in soil. It must be remembered however that the latter workers ensured closer contact by reducing soils to the consistency of a paste, a process which was thought undesirable in the present work, where the soil materials had a high content of undecomposed organic residues.

The method did not reveal statistically significant differences between the soil layers in their effects on mycelial extension. The results in Fig. 1 suggest however, that further work will show inhibitory effects on *T. viride* and *M. hiemalis* in the H and A layers. It also seems probable that *M. ramannianus* will prove to be retarded by some environmental factor common to all the soil layers tested.

In the test environment the factors most likely to cause a retardation are pH, aeration and diffusible inhibitors. Differences in pH are probably not responsible, since pH values for all the layers are very similar (Table 1). The sand control has a higher pH (5.7) but this will not have favoured extension of *M. ramannianus*, the growth optimum of which is given by MÜLLER (1941) as 3.3—4.1.

Aeration in the test environments did not exactly match that in the undisturbed soil; but poor aeration is probably not a cause of the inhibition, since BURGES and FENTON (1953) found that neither *T. viride* nor *M. ramannianus* was adversely affected by soil atmospheres high in carbon dioxide. *M. hiemalis* has not been studied in this respect.

A diffusible inhibitor provides the most likely explanation of the inhibitions found. Pure culture studies have shown (KNOWLES and LAISHLEY,

1959; TOPPS and WAIN, 1957) that fungi are inhibited by exudates from higher plants, and it is probable (BRIAN, 1957) that antibiotic substances are also widely produced in soil. The mycostasis studied by DOBBS and his co-workers (DOBBS and HINSON, 1953; DOBBS and BYWATER, 1957; DOBBS, BYWATER and GRIFFITHS, 1959; DOBBS and GRIFFITHS, 1960) is attributed to microbial activity.

Combining results with those in Table 2, certain ecological conclusions may tentatively be drawn about the five fungi. *T. viride* is most active in the L and F layers, where it is present in a high concentration; its activity in lower layers is probably reduced. *M. hiemalis* is present in smaller concentrations, but is also most active in the upper layers. *M. ramannianus* shows a distinctly lower rate of spread than the above two, but it is H and A layers that have no particular adverse effect upon it; this species is known to be abundant in the mineral layers of acid soils (HEPPLE, 1960). Both *C. herbarum* and *P. fecundissimum* are known to be restricted to the upper layers. It does not appear that diffusible substances are responsible for this restriction.

This is a report of work in progress, and the author is aware that more requires to be done before the usefulness of the method outlined can be regarded as proved.

Summary

Mycelial extension of five litter fungi was measured over sterile oak leaves, buried in soil inside cellophane covers. Preliminary estimates in four soil layers showed inhibitions, compared with a sand control, varying from nil to 50 %. H layer and A layer material was inhibitory to *Trichoderma viride* and *Mucor hiemalis*, while all soil layers inhibited *Mucor ramannianus*. These effects are discussed and tentatively referred to diffusible inhibitors. Inhibition of the remaining two species, *Cladosporium herbarum* and *Polyscytalum fecundissimum*, was not indicated.

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Discussion

T. F. HERING: *Mucor ramannianus* shows non-linear extension, nearly all the growth taking place in the first four days. The daily extension rate as calculated was much too low and is in fact of the same order as those of *Trichoderma viride* and *Mucor hiemalis*.

The results, including additional data for the summer 1962, have been analysed statistically by Mr. P. Holgate of the Biometrics Section of the Nature Conservancy. *Trichoderma viride*, *Mucor hiemalis* and *M. ramannianus* all showed significantly lower rates of extension in all soil layers than in the sand control. In addition extension of *M. hiemalis* in the L plus F layers was significantly higher than in the H plus A layers.

G. J. F. PUGH: I have obtained similar results isolating fungi from leaf litter. *Mucor hiemalis* and *Cladosporium herbarum* were most frequently isolated from newly dead litter and less frequently with increasing age of the litter. *Mucor ramannianus* was most common on older litter, more than two years old. The isolation of *Trichoderma* differed, however, as it was common on newly dead litter and again on very old litter. Presumably this was caused by different strains of *T. viride*.

W. GAMS: Fungistasis is a very important phenomenon in soil mycology, but also a very complex one. Dr. Hering is feeding his fungi on leaves in the same time as he is observing fungistatic effects. What can be observed, is only the balance of these two actions. I can see little prospect in such an approach, because the different effects cannot be separated.

T. F. HERING: The sterile leaf material supplied in the growth test should be sufficient to overcome large effects on activity through external nutritional factors.